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Hybrid Anisotropic Plasmonic Nanomaterials as Nanomedicine for Combinational Chemodynamic and Photothermal Cancer Treatment

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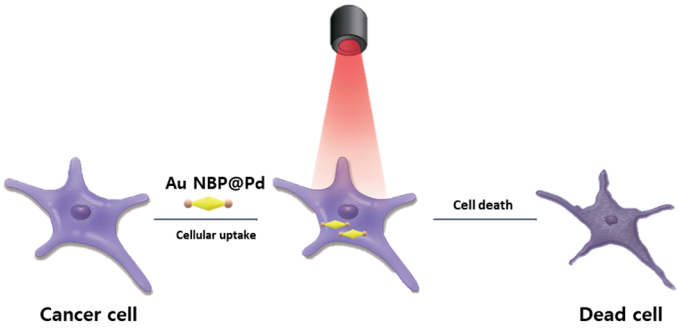
Abstract: We propose the application of a unique gold and palladium bimetallic hybrid plasmonic nanostructure as a biomimetic therapeutic agent for the photothermal and photo-enhanced chemodynamic therapy. Localized surface plasmon resonance (LSPR) induced hot carriers could affect the overall biomimetic process, eventually promote the therapeutic efficacy and overcome the limitation of conventional phototherapy.

1. Introduction

Noble plasmonic metal based nanostructures have received increasing attention due to their intrinsic LSPR properties, which can generate energetic electrons and holes, and sufficient heat to induce photothermal treatment[1]. Generated reactive electrons and holes have also been proposed to participate and enhance the performance of catalytic reactions occurring at noble metal sites. In particular, the noble metal can mimic the enzyme to induce enzymatic reactions such as peroxidase, oxidase, etc[2]. In this study, we introduce a bimetallic plasmonic nanostructure[3], consisting of anisotropic gold nanobipyramid (Au NBP) decorated with palladium nanoparticles (Pd NP), which showed enhanced biomimetic reaction to generate reactive oxygen species. In addition, a photothermal effect could be simultaneously exploited due to the intrinsic LSPR[4], leading to synergistic and effective photothermal and chemodynamic therapy. The overall therapeutic process is illustrated in **Scheme 1**.



Scheme 1. Schematic diagram of the overall *in vitro* therapeutic process under laser irradiation.

2. Material and Methods

Materials and Synthesis

Gold(III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$; ≥99.9% trace metals basis), trisodium citrate dihydrate, sodium borohydride ($NaBH_4$, 99%), hexadecyltrimethylammonium bromide (CTAB, ≥98%), silver nitrate ($AgNO_3$, ≥99.0%), ascorbic acid (AA), hexadecyltrimethylammonium chloride (CTAC, ≥98.0%), 3,3′,5,5′- Tetramethylbenzidine (TMB, ≥99.0%), terephthalic acid (TA, 98%), 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA, ≥97%), phosphate buffered saline tablet (PBS), sodium acetate (>99%), hydrochloric acid (HCl, ACS reagent, 37%), glacial acetic acid (pharmaceutical secondary standard) were all purchased from Sigma-Aldrich. Ammonia solution ($NH_3 \cdot H_2O$, 30%) was purchased from Daejung chemical. Hydrogen peroxide (H_2O_2, 30%) was purchased from Junsei Chemical Co. Ltd. The solvents were used as received and without any further purification.

Synthesis of Au NBPs.

The gold nanobipyramids were synthesized by a seed-mediated method. The seed solution was prepared by mixing $HAuCl_4$ (0.01 M, 0.125 mL) and trisodium citrate aqueous solution (0.01 M, 0.25 mL) in water (9.625 mL) with a freshly prepared, ice-cold $NaBH_4$ aqueous solution (0.01 M, 0.15 mL) in a plastic tube. After inversion mixing for 10 s, the resultant seed solution was colored brownish-yellow and kept at room temperature for 2 h before use. The growth solution was prepared with sequential addition of $HAuCl_4$ (0.01 M, 2 mL), $AgNO_3$ (0.01 M, 0.4 mL), HCl (1 M, 0.8 mL), and ascorbic acid (0.1 M, 0.32 mL) into a CTAB solution (0.1 M, 40 mL). After the solution turned colorless, the seed solution (0.6 mL) was then injected into the growth solution, followed by gentle inversion mixing for 10 s. The reaction solution was left undisturbed overnight at room temperature.

Synthesis of the palladium-tipped Au nanostructures

The purified Au nanostructures (optical density = 0.7) were centrifuged at 9000 rpm for 15 min. The precipitate was redispersed into a CTAC solution (8 mL, 0.8 M), followed by the subsequent addition and mixing of $AgNO_3$ (40 μL, 0.01 M) and ascorbic acid (20 μL, 0.1 M) under gentle stirring. The mixture solution was incubated in an oven (60 °C) for 4 h. During this process, Ag was overgrown on the Au nanostructures to form a Au@Ag nanostructure. The resultant sample was centrifuged three times at 7500 rpm for 15 min. The precipitate was redipersed into a CTAB solution (2 mL, 3 mM). The formation of metal nanoparticles at the tips of the Au nanostructures was carried out by the sequential addition of H_2PdCl_4 (52.4 μL, 1 mM) and ascorbic acid (52.4 μL, 10 mM) under gentle stirring overnight at room temperature. The resultant sample was centrifuged at 7000 rpm for 10 min. The precipitate was redispersed into 3 mM CTAB solution for further use.

Measurements

Transmission electron microscopy (TEM) images were collected using a JEOL JSM2100-F microscope operated at 100 K. Elemental analysis and mapping were accomplished by energy dispersive X-ray spectroscopy (EDX) using a JEOL JEM-3011 at an accelerating voltage of 200 kV. Scanning electron microscopy (SEM) images were taken using a JEOL JSM6700-F. UV−vis absorbance spectra measurements were conducted on a Varian Cary5000 UV−vis−NIR spectrophotometer. Cell viability tests were done using an Infinite M200 PRO micro plate reader. Photoluminescence (PL) spectra were collected using a PerkinElmer LS 55 spectrofluorimeter at room temperature. The 808 nm NIR laser source from Hi-Tech Optoelectronics Co. Ltd was used to induce photothermal and photodynamic effects.

3. Results

Collected morphological and optical properties were depicted in **Figure 1**. Transmission electron microscopy (TEM) images and energy dispersive X-ray spectroscopy (EDX) analysis demonstrated that the

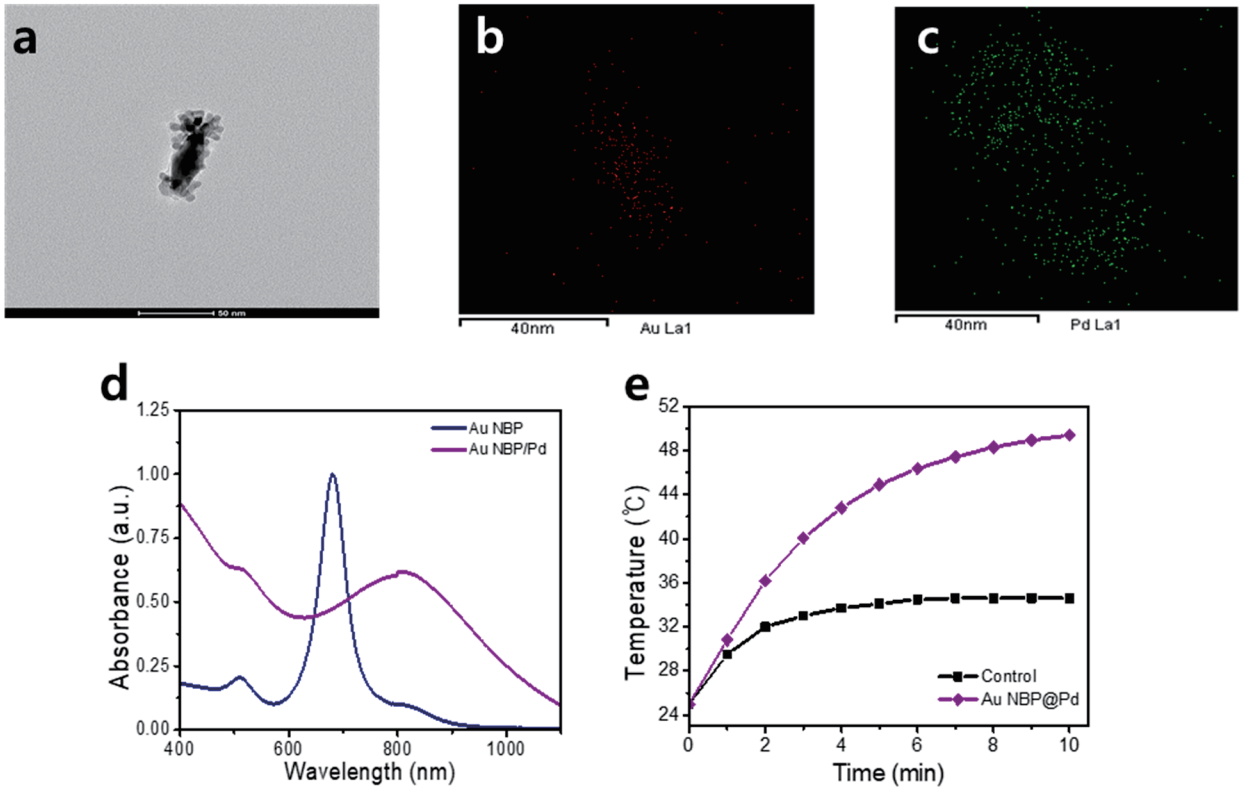


Figure 1. (a) TEM image of Au NBP@Pd, (b-c) EDX mapping data of (b) Au element (c) Pd element, (d) UV-vis spectra of Au NBP and Au NBP@Pd, and (e) time-dependent temperature arising profiles of control (DI-water) and Au NBP@Pd under 808 nm (2W/cm^2) laser irradiation.

bimetallic nanostructures (Au NBP@Pd) were well prepared (**Figure 1a-c**). In **Figure 1d**, the LSPR peaks of Au NBP@Pd were slightly red-shifted against those of the bare Au NBP due to the decoration of metal Pd NPs due to a change of the reflective index of the Au surroundings. The LSPR-induced photothermal properties were confirmed by time-dependent temperature rising profile (**Figure 1e**). Compared with bare Au NBP, the plasmonic coupling effect between Au and Pd was reflected by a resulting higher temperature.

To confirm the enhanced biomimetic reaction, a 3,3',5,5'-Tetramethylbenzidine (TMB)-based colorimetric assay was performed. In **Figure 2a**, characteristic absorption peaks (652 nm) observed with Au NBP@Pd and H_2O_2 demonstrated that our bimetallic nanostructure could lead to the biomimetic process. Most importantly, under laser irradiation, the overall activity of the biomimetic process could be significantly enhanced based on an effective formation of reactive oxygen species (ROS) due to the generation of energetic carriers. To verify the effective generation of ROS, we conducted terephthalic acid (TA) assays and EPR analysis. As shown in **Figure 2b-c**, in the case of Au NBP@Pd with H_2O_2 under laser irradiation, superior fluorescence and electron paramagnetic resonance (EPR) signals were observed, suggesting an effective generation of ROS ascribed to the generated hot carriers capable of enhancing and participating in the biomimetic reaction.

To investigate the promise of Au NBP@Pd in biomedical application, we evaluated corresponding *in vitro* properties with Human Glioblastoma Cells (U87MG) (**Figure 3**). To assess the cellular uptake

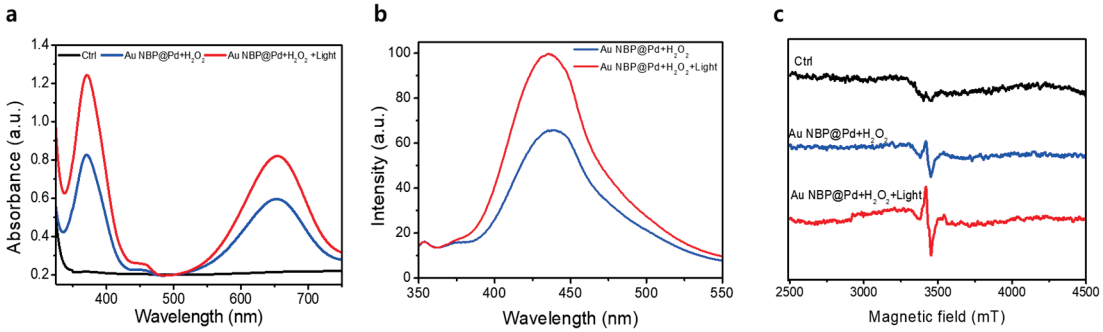


Figure 2. (a) Uv-vis spectra of TMB in the presence of Au NBP@Pd and H_2O_2 under laser. (b) Photoluminescence spectra of TA with Au NBP@Pd with H_2O_2 in the presence and absence of laser irradiation. (c) EPR signals of ·OH generated by Au NBP@Pd with H_2O_2 in the presence and absence of laser irradiation.

ability, we collected dark field images of Au NBP@Pd@GC. As shown in **Figure 3a**, bright spots were observed inside the cells, which were attributed to the effective cellular uptake properties of Au NBP@Pd@GC. Biocompatibility and therapeutic efficacy of Au NBP@Pd@GC were confirmed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. As shown in **Figure 3b**, no significant cytotoxicity was observed without laser irradiation, confirming a suitable biocompatibility in nanomedicine. In **Figure 3c**, effective cytotoxicity was shown with laser irradiation, demonstrating that the enhanced biomimetic reaction and synergistic photothermal effect could significantly eliminate the cancer cells. Live and dead cell assay (**Figure 3d**) further corroborated the aforementioned MTT results. Finally, an effective *in vitro* ROS generation was confirmed by DCFH-DA assay (**Figure 3e**). Prominent green fluorescence was observed in the collected images with Au NBP@Pd@GC and H_2O_2 under laser irradiation, inferring an enhanced intrinsic biomimetic activity by generated energetic electrons and holes. Taken together, our bimetallic nanostructure appears to be promising as a nanomedicine for curing the cancer by enhanced intrinsic biomimetic properties and synergistic photothermal effect.

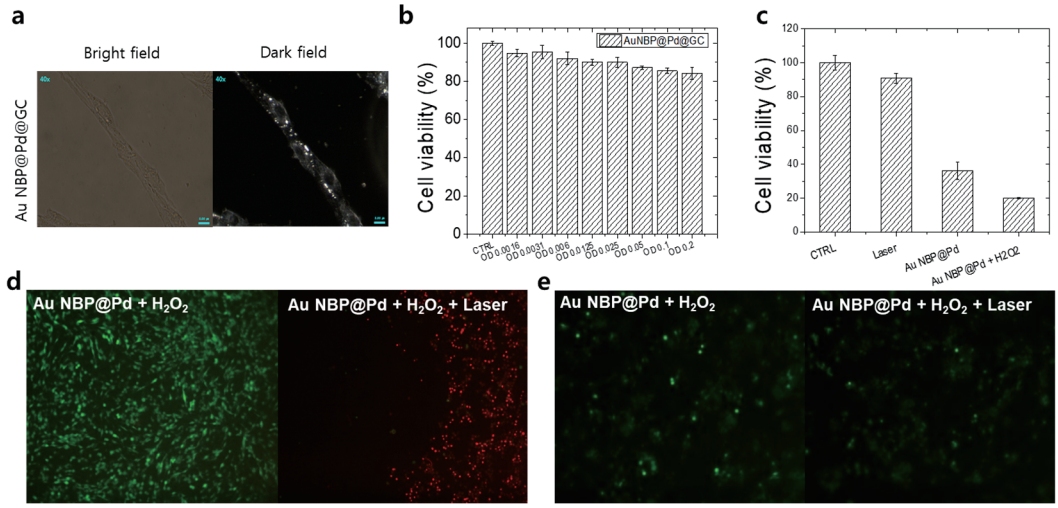


Figure 3. (a) Bright and dark field images incubated with Au NBP@Pd@GC, (b) cell viability by MTT assay treated with different concentrations of Au NBP@Pd@GC, (c) MTT assay for various samples, (d) live and dead cell assay, green: Calcain, red: PI and (e) DCFH-DA assay for investigating *in vitro* ROS level.

4. Conclusion

In this report, we developed unique hybrid structures for cancer treatment by LSPR-enhanced intrinsic biomimetic properties and photothermal effect. We confirmed the generation of LSPR-induced energetic carriers, which enhanced the intrinsic catalytic properties of the incorporated Pd NPs, leading to superior generation of ROS. In addition, the LSPR-induced local heat was proved to lead to synergistic photothermal therapy in the elimination of cancer cells.

Our work demonstrated the potential ability of noble metal-based hybrid nanostructures as a LSPR-derived photothermal and chemodynamic nanomedicine in near-infrared (NIR)-based noninvasive cancer therapy.

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